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Off-host oviposition by two fritillary species (Nymphalidae, Argynninae) and its relation to egg predation

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Abstract Off-host oviposition of *Argyreus hyperbius* and *Argynnis paphia* was investigated. We obtained data on oviposition sites in the caged condition and on predation ratios of eggs exposed to a natural habitat. *A. hyperbius* oviposited both on and off the host plant with a significantly higher density on it. They laid more eggs on forbs than on dead leaves or stones with a tendency for the number to increase nearer the host plant. *A. paphia* oviposited at higher levels of the net cage far from the short host plant. As the predation ratio of *A. paphia* eggs was higher at lower levels, it was hypothesized that this species evolved a habit to oviposit at higher levels to avoid ground predators. For the occasional off-host oviposition by *A. hyperbius*, possibilities of predation and transition of oviposition habit are discussed.

Key words *Argynnis*, *Argyreus*, egg, fritillary, oviposition, predation.

Introduction

Females of butterflies usually lay eggs on their larval food plants, but some species are known to oviposit off their host plants (Dethier, 1953, 1959; Wiklund, 1984; Singer 1984). Fritillaries (Nymphalidae) are reported to be such species (Frohawk, 1924; South, 1941; Magnus, 1950; Wiklund, 1984; Kopper *et al.*, 2000). Some Japanese fritillaries oviposit off the food plants, and some on and off them (Fukuda *et al.*, 1983). The reason for the off-host oviposition by species, which use herbaceous host plants and overwinter in the egg stage, was supposed to be because the host plants wither during the winter and eggs might be blown away with leaves (Wiklund, 1984). However, some fritillaries that overwinter in the larval stage on host plants that do not wither in winter, also have an off-host oviposition habit (ref. Table 2), to which the above explanation does not apply. For these species, a strategy to avoid egg predators or parasitoids has been proposed (Benson *et al.*, 1976; Wiklund, 1984).

We conducted experiments about oviposition and predation of eggs of fritillaries, constructing an artificial environment to determine exact oviposition sites and egg predation ratios. We examined two species of fritillaries, *Argyreus hyperbius* (L.) and *Argynnis paphia* (L.). From fragmentary reports, the former species is known to oviposit on and off the host plant, and the latter to oviposit off the host plant (Fukuda *et al.*, 1983).

Materials and methods

Outline of the species examined

The Indian fritillary, *Argyreus hyperbius* (L.), is widely distributed from the tropics to the warm temperate zone in the Old World, and Japan is the northern limit. They are widely seen in the sunny grassy ground around woods, cultivated fields, and suburban gardens in warm regions in Japan. *Argyreus hyperbius* is multivoltine, and oviposits from spring to autumn. Eggs are laid singly on the host plant, other plants, sticks, stones or soil (Fukuda *et al.*, 1983). They overwinter as undormant larvae or pupae. The host plants of *A. hyperbius*

are some species of violets.

The silver-washed fritillary, *Argynnis paphia*, is distributed throughout the temperate Palaearctic region, and widely in woodland clearings and forest roads in Japan. They are univoltine, and lay eggs from August to October. They overwinter as first instars eating a little their egg chorion after hatching, or sometimes overwinter as eggs. The larvae start eating the host plant the next spring. The adults emerge from June to August, and then estivate in the middle of summer in the warm region (including our experimental site). Females are observed to deposit eggs singly among crevices in the bark of tree trunks of Japanese cedar, *Cryptomeria japonica*, Cherry, *Prunus* sp., and on other substrates around the host plant (South, 1941; Fukuda *et al.*, 1983; Wiklund, 1984). The host plants of *A. paphia* are some species of violets.

Collection site of females

A. hyperbius females used in this experiment were collected in the cities of Ono, Kasai and Kobe in Hyogo Prefecture, Japan. *A. paphia* females were collected in Mt. Rokko in Kobe. Both were within 35 km of the experimental sites.

Experimental site

The experiment on *A. hyperbius* was conducted in September and October 2005 in a house garden in Kobe City (135°04′N, 34°67′E) where ordinary garden plants and weeds were grown; mainly bugle, *Ajuga reptans* (L.); mint, *Mentha* sp.; honewort, *Cryptotaenia japonica* Hassk.; common crab grass, *Digitaria ciliaris* (Retz.) Koeler.; and also violet *Viola mandshurica* W. Becker, the larval food plant of this species which the adult females sometimes visited to oviposit. The average temperature at this site in the experimental period was 23.2°C.

The experiment on *A. paphia* was conducted in September and October 2005, and October 2006 in secondary mountain forest in The Kobe Municipal Arboretum (135°18′N, 34°74′E) halfway up Mt. Rokko. Flora of this site was native to secondary vegetation with trees principally composed of Japanese Red Pine *Pinus densiflora* Sieb. et Zucc. and a species of oak *Quercus serrata* Thunb., and forbs of chocolate vine *Akebia quinata* (Thunb.) Decaisne, Ground Ivy *Glechoma hederacea* L., along with the host plant, *Viola grypoceras* A. Gray, of this butterfly species. The average temperature at this site in the experimental period was 19.9°C.

Oviposition experiment

We prepared a cylindrical wire net cage (120 cm in diameter, 90 cm high, Fig. 1A) and planted one host plant at the center of the floor; *Viola mandshurica* W. Becker for *A. hyperbius*, and *Viola grypoceras* A. Gray for *A. paphia*. Both plant species were short (<15 cm) perennial and the most common in the experimental areas. The former plant species does not wilt throughout the year, and the latter species wilts in cold regions, but not in warm regions (including our experimental site). In the experiment on *A. hyperbius*, we prepared 3 types of substrates, naturally grown forbs, dead leaves and stone (4–10 cm in dia.) (Fig. 1B). In the experiment on *A. paphia*, we left the ground in the natural condition and stood a branch of Japanese cedar *Cryptomeria japonica* D. Don 22 cm from the center (Fig. 1C).

We released one female into the cage at 9:00 AM and took it out at 3:00 PM. After recovery of the female, we closely checked the inside of the cage to record numbers and positions of

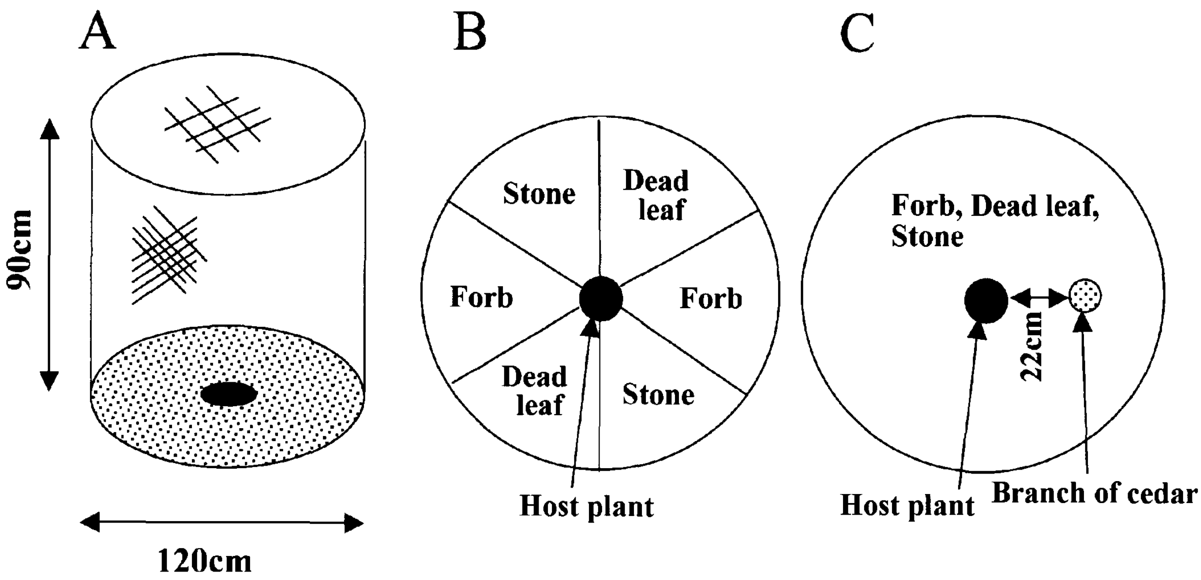

Fig. 1. The wire net cage (A) and arrangement of test substrates (B, C). In the experiment on *A. hyperbius*, three types of substrates were arranged in an equal area with the food plant, *Viola mandshurica*, at the center (B). Forb is a live natural-standing short plant. In the experiment on *A. paphia*, the floor was left as it was without arrangement, containing many forb plants, dead leaves and stones, except for a standing branch and the food plant, *Viola grypoceras*, at the center (C).

eggs laid.

Egg predation experiment

Eggs laid by captured females were used; females were put separately in transparent plastic cups (15 cm dia., 15 cm high) with host plant leaves, and left in a sunny room for several hours.

The eggs obtained were pasted on a piece of pasteboard (1 × 3 cm). For *A. hyperbius*, they were attached to each of 16 wires at 5 cm intervals over a distance of 60 cm and placed horizontally 2 cm above the ground with the host plant at one end (Fig. 2A). On that host plant, eggs were attached. For *A. paphia*, we prepared 7 horizontal wires just as for *A. hyperbius* without attaching eggs on the host plant, and also 9 vertical wires on which pasteboards with eggs were attached at 10 cm intervals to a distance of 150 cm, and fixed vertically to the tree trunks of Chinese fir *Cunninghamia lanceolata* (Lamb.) Hook., Japanese hackberry *Celtis sinensis* Pers., and giant dogwood *Cornus controversa* Hemsley, 60 cm distant from the food plant (Fig. 2B).

Two days later, we checked the eggs for predation and parasitization.

Data analysis

Statistical analyses were performed with StatView 5.0 (SAS Institute).

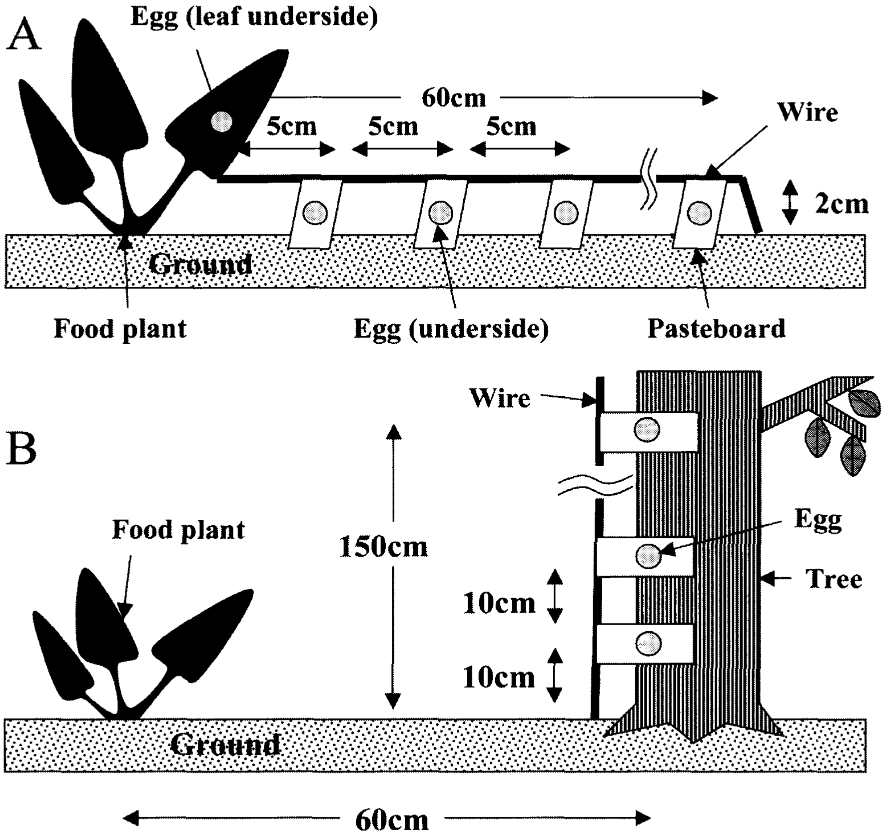

Fig. 2. The horizontal experiment of predation pressure on *A. hyperbius* and *A. paphia* eggs (A) and the vertical experiment of predation pressure on *A. paphia* eggs (B). Eggs were attached at 5 cm intervals above the ground (A) or at 10 cm intervals along the tree trunk (B).

Results

Oviposition site

In *A. hyperbius*, 7 out of 12 females laid 129 eggs in total. They laid 34 eggs on the host plant and 95 eggs on the other substrates, forbs and dead leaves. The egg density on the host plant was higher than that on the other substrates (Table 1). The egg density tended to increase nearer the host plant (Fig. 3A). Apart from the host plant, they oviposited most frequently on forbs, secondly on dead leaves, and no eggs were found in the stone section (Fig. 4).

In *A. paphia*, 5 of 17 females laid 321 eggs in total. They oviposited mostly on the ceiling and the side of the wire net cage, some on the branch of Japanese cedar and not at all on the host plant (Table 1) or near the ground. More eggs were laid at higher levels on the net side wall (Fig. 3B).

Predation pressure

Fifty-three out of 155 eggs were eaten in the horizontal experiment of *A. hyperbius*. Predation ratios were not different between eggs on the host plant and those on the pasteboards (40 % vs 34 %, $P > 0.8$, x^2-test). There was no tendency for eggs to be eaten nearer to or farther from the host plant (Fig. 5C).

Forty-eight out of 69 eggs were lost in the horizontal experiment of *A. paphia* with no sig-

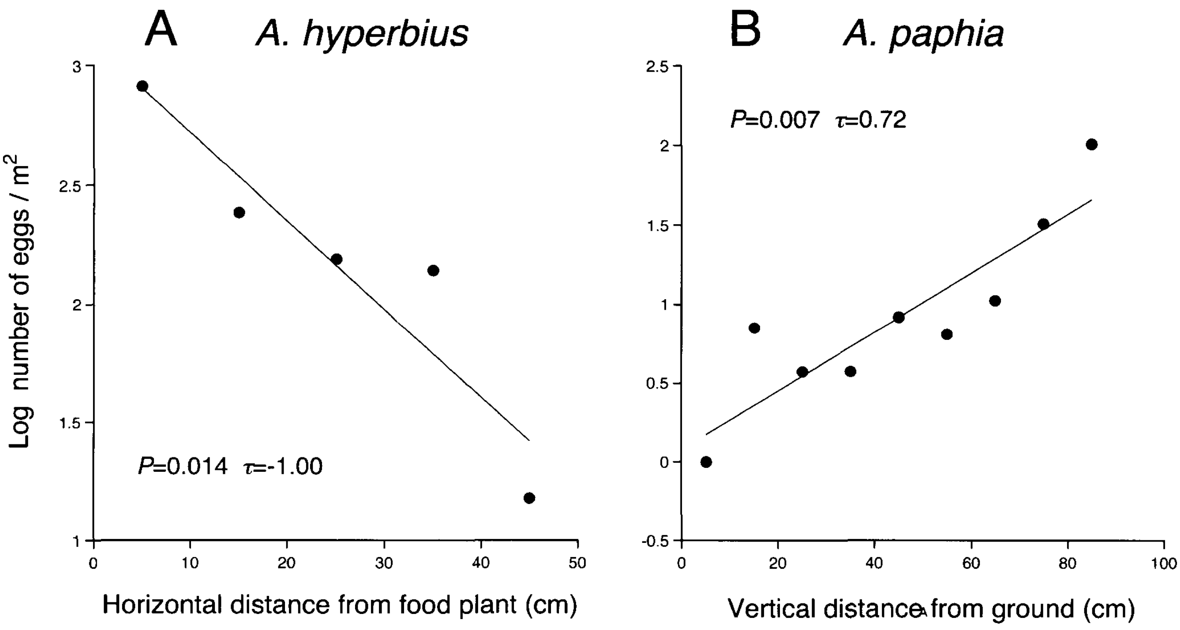

Fig. 3. Egg density (/m^2) and horizontal distance from the food plant for *A. hyperbius* (A) and vertical distance from the ground for *A. paphia* (B). *P*-values are based on a Kendall's rank correlation test.

nificant tendency depending on the position of eggs (Fig. 5A). In the vertical experiment of this species, 45 out of 110 eggs were lost, and they tended to be distributed at lower levels (Fig. 5B).

The predation ratios differed between the species examined; in the horizontal experiment, it was 34 % for *A. paphia* and 70 % for *A. hyperbius* ($P < 0.0001$, x^2-test). Thus, the eggs of *A. paphia* were eaten 2.1 times as often as those of *A. hyperbius* near the ground.

Many eggs were eaten together with their shells, but in some cases only the contents were eaten, leaving the shell. No parasitoid emerged from the uneaten eggs.

Discussion

In our experiment, *A. paphia* laid eggs completely off the host plant (Table 1). This confirmed the oviposition behavior observed in nature for Japanese (ref. Fukuda *et al.*,

Table 1. Comparison of egg density (/m^2) on and off the host plant.

Argyreus hyperbius			*Argynnis paphia*		
Individual	Host plant	Others	Individual	Host plant	Others
H1	1410	0.9	P1	0	0.9
H2	0	0.4	P2	0	20.5
H3	4950	3.0	P3	0	8.0
H4	2120	2.7	P4	0	17.2
H5	354	1.6	P5	0	10.3
H6	2830	1.4			
H7	354	6.9			
Average	1720	2.4		0	11.4
P (*Z*)	0.028 (-2.197)			0.043 (-2.023)	

P-values are based on Wilcoxon's signed-ranks test.

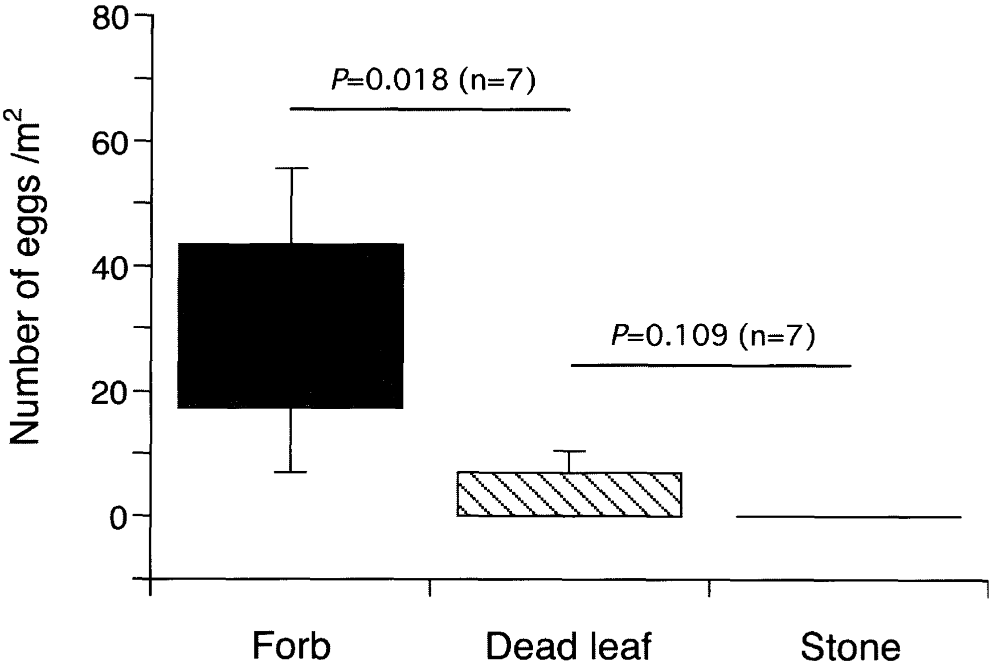

Fig. 4. Comparison of egg density among oviposition substrates. *P*-values are based on a Mann-Whitney *U*-test.

1983) and also European butterflies (Wiklund, 1984; South, 1941). This oviposition habit seems to be related to the life history of this species: they do not eat for a while after hatching because they enter winter diapause. All Japanese fritillaries with this type of life history oviposit off the host plant (Table 2).

In our experiments, *A. paphia* laid more eggs at higher levels (Fig. 3B), and egg predation pressure on this species was lower there (Fig. 5B). Thus, they tend not to oviposit on the host plant which is short and grows near the ground. The average predation ratio of *A. paphia* eggs in the experiment carried out in the forest was 69%, whereas that of *A. hyperbius* eggs in the experiment in the garden was 33%, suggesting that high predation pressure in the forest forces the sylvan *A. paphia* to oviposit at higher levels. The fact that forest fritillaries tend to oviposit at high levels, whereas meadow fritillaries do so near the ground (Fukuda *et al.*, 1983, Table 2) supports the idea that the oviposition habit of fritillaries is affected by predation pressure.

In our experiment, we did not succeed in identifying egg predators. No egg predators for fritillaries in the Heliconiinae are listed, though slugs and scorpion fly larvae are described for the Nymphalinae in the encyclopedia by Fukuda *et al.* (1983). As a preliminary observation, we put *A. paphia* eggs together with the common pill bug *Armadillidium vulgare* in a plastic box for one night with the result that all eggs were consumed. The eggs from which only the contents were sucked out in our experiment may have been attacked by mites (Acari), and assassin bugs (Reduviidae, Hemiptera) which are known as predators of other nymphalid butterfly eggs (Fukuda *et al.*, 1983).

A. hyperbius oviposited both on and off the host plant (Table 1). We suppose this is because *A. hyperbius* does not hibernate in the egg or the first-instar larval stage, but begins eating the host plant soon after hatching; thus, they have to oviposit on or near the host plant. All the Japanese fritillaries with this type of life history lay eggs both on and off the host plant (Table 2).

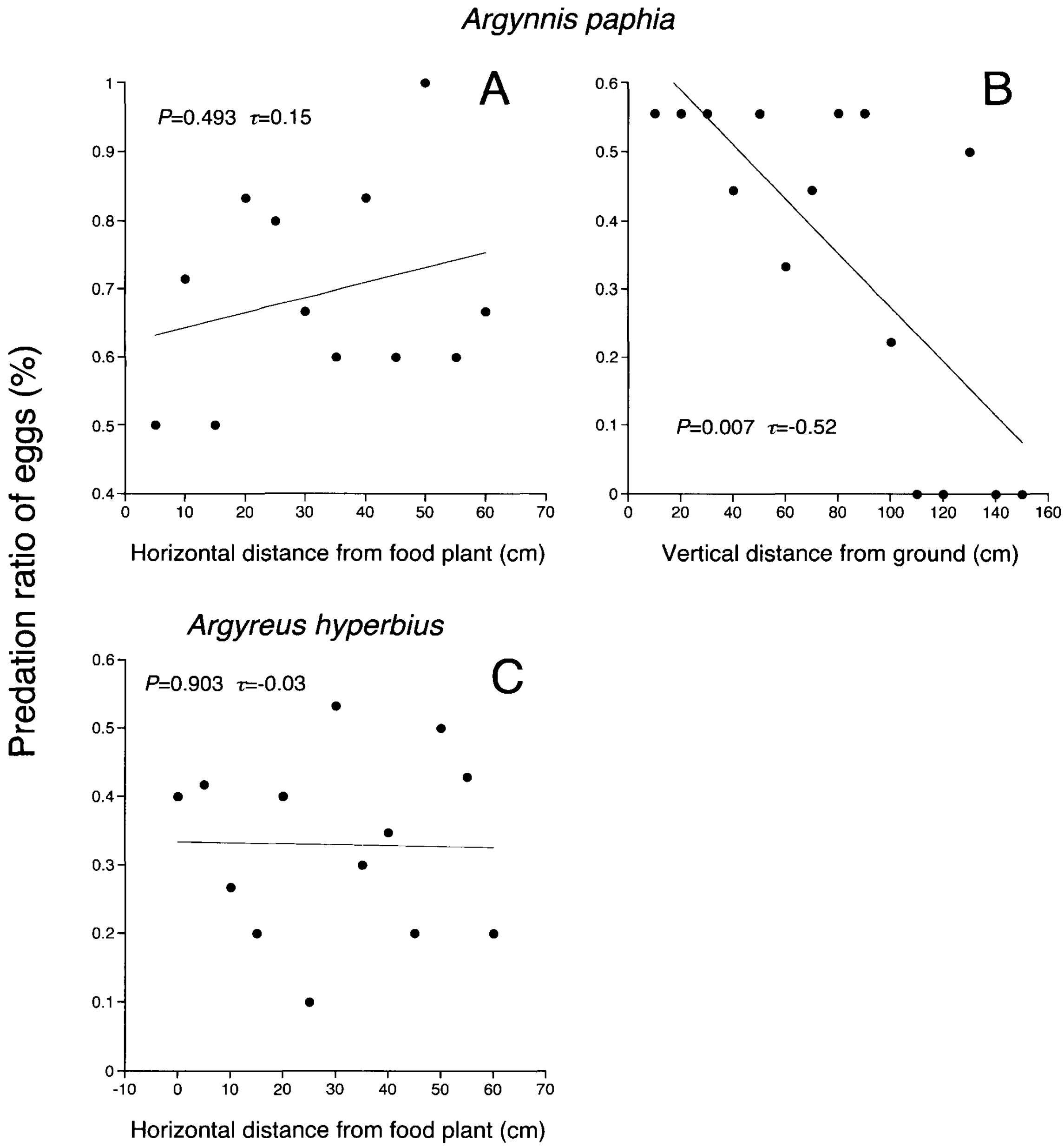

Fig. 5. Predation ratio of eggs and horizontal distance from food plant (A, C) or vertical distance from the ground (B). *P*-values are based on a Kendall's rank correlation test.

In our experiments, *A. hyperbius* laid more eggs nearer the host plant. From the physiological point of view, this could be explained by the fact that they need to touch the host plant sometimes during an oviposition sequence (Fukuda *et al.*, 1983), and thus, the higher frequency of stimuli near the host plant seems to excite them to lay eggs there. From the adaptive viewpoint, the nearer the oviposition site is to the host plant, the easier the hatching larvae can reach it. Predation pressure independent of distance from the host plant (Fig. 5C) would not hinder the butterfly from this oviposition habit.

Why do they oviposit not only on the host plant, but also off the host plant? One hypothesis is that the host plant surface is a dangerous area, because some predators may use the host plant as a clue to their potential prey, as Benson *et al.* (1976) and Wiklund (1984) pointed out. A parasitoid *Cotesia glomerata* (L.) (Braconidae) of the cabbage white *Pieris rapae*

Table 2. Summary of overwintering stage, oviposition habit and habitat of Japanese fritillaries (Argynnini) (data from Fukuda *et al.*, 1983).

Species	Overwintering stage[a]	On-host oviposition	Off-host oviposition		Habitat[b]
			near ground	tree	
Clossiana fleija	4	++	+	–	H
Clossiana thore	2 4	++	++	–	F
Clossiana iphigenia	2 3	+	++	–	F.M
Brenthis daphne	YL	++	++	–	M
Brenthis ino	YL	++	+	–	M
Argyronome laodice	E1, 1	–	++	–	M
Argyronome ruslana	E1, 1	–	++	++	G, F
Argynnis paphia	E, 1	–	+	++	F
Nephargynnis anadyomene	1	–	++	++	F
Damora sagana	1	–	++	++	F
Fabriciana adippe	? probably E, 1)	–	++	–	M
Fabriciana nerippe	1	–	++	–	M
Speyeria aglaja	E1, 1	–	++	–	M
Argyreus hyperbius	undormant E, L, P	++	++	–	O

[a] E: egg, E1: 1st instar inside egg, YL: young larva, L: larva, 1–4: larva of that instar, P: pupa. [b] H: heath, F: forest, M: meadow, G: grassland, O: open land.

(L.) (Pieridae), for example, uses the plant volatile from the larval bite as a key to finding host larvae (Sato 1979). As for egg parasitoids of Lepidoptera, many trichogrammatid wasps are known, and *Trichogramma brassicae* Bezdenko were shown to perform a longer search on the leaf of the plant species on which they emerged (Bjorksten & Hoffman, 1998): this wasp species is recorded as attacking some other fritillaries, *Fabriciana adippe* (Denis & Schiffermüller) and *F. niobe*, (L.) (Noyes, 2003). Though no parasitoid emerged in this experiment, *Trichogramma* sp. was confirmed from *A. paphia* eggs in our preliminary observation made in May 2005. Thus, the predation hypothesis for *A. hyperbius* needs to be tested further.

Another hypothesis about occasional oviposition off the host plant is that *A. hyperbius* is in transition from a life history involving oviposition totally off the host plant to one totally on the host plant. The ancestor of fritillaries belonging to the Heliconiinae seems to be temperate to boreal in origin and to have had a totally off-host oviposition habit. This is inferred from the fact that many fritillary species are distributed in temperate to boreal areas and are univoltine with this oviposition habit. *A. hyperbius* may have extended its distribution to southern warm environments, abbreviating winter diapause, acquiring multivolinism, and attempting to change oviposition habit to the on-host type. However, this scenario could not explain why there are some fritillaries with an on-host oviposition habit even though living in a cool environment, such as *Clossiana freija* (Thunb.), *C. thore* (Hübner), and *C. iphigenia* (Graeser) (Table 2). Thus, the transition hypothesis is tentative at present. Detailed data about the oviposition habits, predation pressure and the phylogenetic relationships of many fritillaries are needed.

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摘　要

ヒョウモンチョウ2種 (タテハチョウ科, ヒョウモンチョウ亜科) による食草外産卵と卵被食との関係 (柴田洋昭・今福道夫)

ツマグロヒョウモン *Argyreus hyperbius* とミドリヒョウモン *Argynnis paphia* の食草外産卵について調べた. 母蝶による産卵場所は, 食草およびそれ以外のものを含むケージの中で, 卵の被食率は野外の自然条件下で調べた. ツマグロヒョウモンは, 食草にも食草外にも産卵したが, 石や枯れ葉よりは生きた植物に多く卵を生み, また食草近くに多く生む傾向を示した. ミドリヒョウモンは食草から離れたケージの上部に産卵した. ミドリヒョウモンの卵の被食率は地表付近で高かったことから, 本種の高所への産卵習性は, 地上捕食者から卵を守るために進化したものと思われた. 一方, しばしば見られたマグロヒョウモンの食草外産卵については, 被食回避や, 産卵習性の移行の可能性を検討した.

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